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Antioxidant Activities of Malaysian *Plukenetia volubilis*

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Abstract: *Plukenetia volubilis* (*P. volubilis*) also known as sacha peanut, inca peanut or mountain peanut belongs to the Euphorbiaceae family and contains antioxidants. In this study, Malaysian *P. volubilis* was used to study of total phenolic content (TPC) and the total of flavonoid content (TFC) along with its antioxidant activity by using 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method. The collected *Malaysian P. volubilis's* dried kernel were separate from its shell to extract its oil by using cold pressed method. The Folin-Ciocalteu method was used to evaluate TPC, and the production of flavonoid-aluminium complex was used to estimate TFC. The ABTS radical cation decolorization assay was used to evaluate the ABTS free radical-scavenging activity. The result shows that the highest inhibition of the *P. volubilis* was 20.31% at 125 ppm and the IC_{50} value of ABTS sample was 4405.96 ppm meanwhile the results obtained for the total flavonoid was 2.50 RE ppm and total phenolic content was 2.90 GAE ppm. It was concluded that the Malaysian *P. volubilis* is a potential source of antioxidants agent. The data is significant for the development of neutraceutical products as alternative and treatment to diseases related to cellular damage due to free radicals.

Keywords: Antioxidant Activity, ABTS method, *Malaysian Plukenitia. volubilis* Cold Pressed Oil, Total Phenolic Content, Total Flavonoid Content

1. Introduction

Plukenitia volubilis (*P. volubilis*) is a green cloured star-shaped fruit with the size between 3 to 5 cm and changes to brown in colour at maturity. The *P. volubilis* is rich in biochemical compositions such as lipids (35–60%) (including ω-3, 6, and 9 fatty acids), proteins (25–30%) (including essential amino acids such as cysteine, tyrosine, threonine, and tryptophan), vitamin E, polyphenols, and minerals [1]. Natural antioxidants are perceived safe, less toxic and beneficial for human health. Exogenous (dietary) antioxidants have long been proven found in plants. It is estimated that two-thirds of the world's plant species have therapeutic value, and nearly all of these have high antioxidant potential. The discovery and subsequent separation of ascorbic acid from plants sparked initial interest in exogenous plant antioxidants [2]. Natural antioxidants, on the other hand, are highly cost and have not been widely commercialized. Due to consumer worries about the safety of synthetic antioxidants, the demand for natural antioxidants has recently grown. Because of their health-promoting qualities, there has been a surge in interest in researching the antioxidants found in some fruits [3].

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Antioxidants are a sort of chemical component found naturally in food that can help the body prevent or reduce oxidative stress. Due to the constant use of oxygen, the body is always generating free radicals. These free radicals cause cell damage in the body, which can result in a range of health problems such as heart disease, diabetes, macular degeneration, and cancer. Antioxidants, which are good free radical scavengers, help to prevent and repair cell damage caused by free radicals. Consuming an antioxidant-rich diet helps the body prevent damage caused by free radicals and may lower your risk of illnesses that interfere with detoxification [4].

Antioxidants also can be found in *P. volubilis*. *P. volubilis* has benefit in the anti-proliferative activity. This activity is about several tumor cell lines which indicates that extracts from *P. volubilis* have the ability to treat cancer [5].There are several method of antioxidant activities used for *P. volubilis* such as ferric chelating, 2,2-diphenyl-1-picrylhydrazyl(DPPH) and also can be determined by utilizing the 2,2- azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) [6].

Although *P. volubilis* has been widely consumed as supplementary food due to its nutritional benefits, however the quality of *P. volubilis* from Malaysia was not extensively studied. Therefore the main objective for this study is to determine the antioxidant activity of *Malaysian P. volubilis* cold pressed oil by using the 2,2- azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) in relation to its total flavonoid and phenolic content.

2. Materials and Methods

2.1 Materials

P. volubilis sample were collected from Kelantan by MABA Intellectual Resources. Chemcials such as folin- ciocalteu (Merck, Germany), sodium carbonate (Merck, Germany), aluminium chloride (Merck, Germany), potassium persulfate (Merck, Germany) and 2,2- azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Merck, Gernamy), ethanol (Merck, Germany), rutin hydrate (Merck, Germany) and gallic acid (Merck, Germany) were readily available in the laboratory. All reagents used were of HPLC grade. Instruments such as UV-Vis spectrophotometer U-3900H (Hitachi, Japan), analytical balance (Sartorius, France), Micro-pipet (Eppendorf, Germany), oil press machine (Dsoilpress, China).

2.2 Extraction of *P. volubilis* Oil Using Cold Pressed Method

The *P. volubilis* were washed thoroughly and were blotted to dryness followed by the removal of the shell part to obtained the kernel. Next, the kernels were pressed by using the cold pressed machine in order to obtain its oil (**Figure 1)**. The extracted oil were stored at 4 ºC before further analysis. The *P. volubilis* sample were used to identify its antioxidant activities.

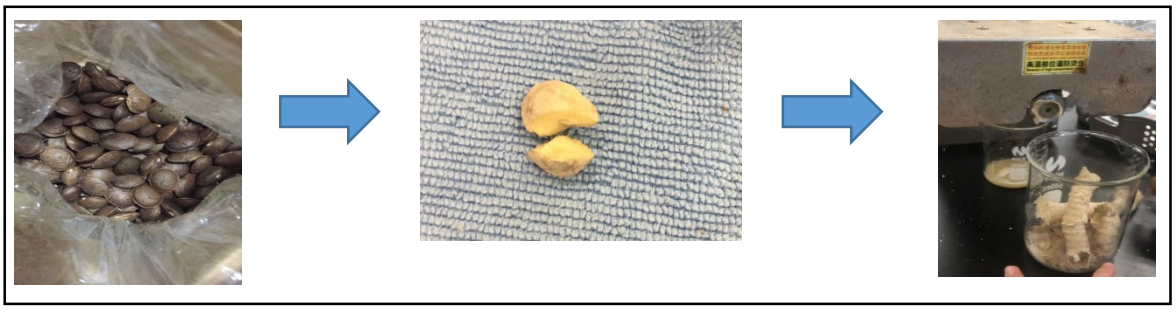

Figure 1: Overall extraction steps of *Plukenetia volubilis* using cold pressed method

2.3 Antioxidant Activity of Cold Pressed *P. volubilis* Oil

The ABTS radical scavenging activity of *P. volubilis* sample was determined by using ABTS radical cation decolorization assay. The ABTS radical cation (ABTS+) was created by combining 7 mm of ABTS solution in water with 2.45 mm potassium persulfate solution in water in a 1:1 (v/v) ratio.The solution was kept in darkness for 16 hours before use. After that the solution was diluted with ethanol until it had an absorbance of 0.7 at 734 nm. For the assay, 2 ml of ABTS working solution was mixed with 100 ml of sample or standard or blank and was incubated for 6 minutes. After 6 minutes of incubation, the UV-Vis spectrophotometer was used to determined the absorbance value at 734 nm. Gallic acid (GA) was used as the positive control and a standard to obtain the standard calibration curve (0 – 0.1 ppm) [7]. The formula in **Eq. 1** was used to compute the percentage of ABTS inhibiton:

$$\text{Percentage of inhibition (\%)} = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}} \times 100\ \%. \qquad \textbf{Eq. 1}$$

2.4 Determination of Total Flavonoid Content (TFC)

A 5 ml of *P. volubilis* was mixed with 5 ml of 2% (w/v) aluminium chloride. Then the solution was incubated for 10 minutes in dark. The absorbance was measured using UV-Vis spectrophotometer U-3900H (Hitachi, Japan) at 430 nm [8]. Rutin was used as the positive control and a standard to obtain the standard calibration curve (0.02 – 0.80 ppm).

2.5 Determination of Total Phenolic Content (TPC)

The TPC of the *P. volubilis* sample was analyzed by using Folin-ciocalteu reagent. The 0.5 ml of the *P. volubilis* sample was mixed with 50% (v/v) of Folin-ciocalteu (2.75mg/ml) and incubated for 5 minutes followed by the addition of 2 ml of sodium carbonate (2% w/v) to terminate the reaction. The solution was mixed thoroughly and incubated for 2 hours in dark. The absorbance was measured using UV-Vis spectrophotometer U-3900H (Hitachi, Japan) at 720 nm [7]. Gallic acid was used as the positive control and a standard to obtain the standard calibration curve (0.02 – 0.10 ppm).

3. Results and Discussion

3.1 Antioxidant Activity of Cold Pressed *P. volubilis* oil

ABTS free radical-scavenging activity of the *P. volubilis* extracts was investigated to determine the antioxidant properties. The finding for the ABTS *P. volubilis* sample are presented as percentage of inhibition against concentration in **Figure 2** while **Figure 3** shows the results for the ABTS gallic acid standard. The comparison of IC_{50} values for ABTS sample and ABTS gallic acid standard are presented in **Table 1**. According to the **Figure 2**, the concentration at 125 ppm has the highest value of inhibition which is 20.31% while the lowest is at concentration 1000 ppm which is 16.25%.

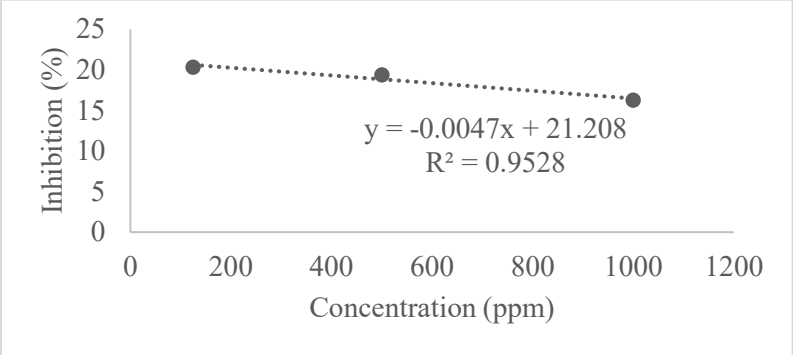

Figure 2: Percent of inhibition for *P. volubilis*

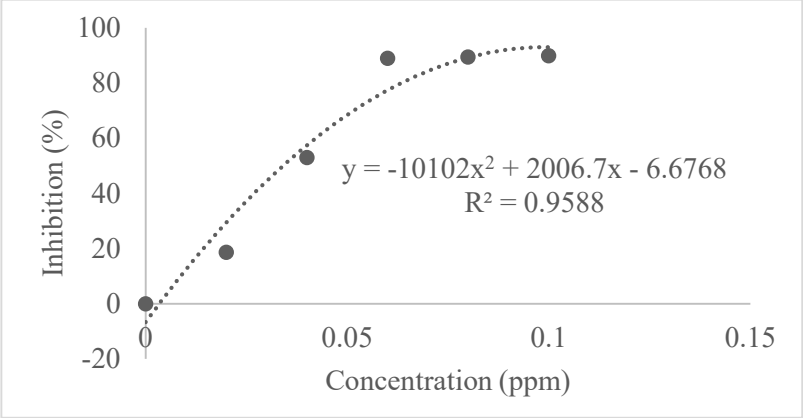

Figure 3: Percent of inhibition for gallic acid

Based on **Figure 3**, the highest inhibition value was at the concentration 0.1 ppm which is 89.84% and the concentration at 0.02 ppm has the lowest value which is 18.59%.

Table 1: The IC_{50} values for ABTS Activity of *P. volubilis* and Gallic Acid Standard

Sample	IC_{50} (ppm)
P. volubilis	4405.96
Gallic Acid Standard	0.17

According to **Table 1**, it's shown that the IC_{50} of *P. volubilis* (4405.96 ppm) was higher than gallic acid standard (0.17 ppm) which means that the antioxidant activity in of *P. volubilis* is lower than gallic acid standard. In the ABTS free radical scavenging assay, the IC_{50} (Half maximal Inhibitory Concentration) value is the sample concentration that can scavenge half of the ABTS free radical. The IC_{50} value is inversely related to the sample's free radical scavenging activity. If the IC_{50} value is lower, the sample will require less quantity to scavenge the free radical. The presence of compounds known as phenolic and flavonoid compounds in the sample contributes to the scavenging activity of free radical.

3.2 Total Phenolic Content (TPC)

Plant phenolics are essential components that contribute to functional quality, color, and taste, as well as serving as singlet oxygen quenchers and free radical scavengers, assisting in the reduction of molecular damage [9]. The gallic acid equivalent (the standard curve: y = 0.2161x-0.1053) was used to express the total phenolic contents in the examined plant extracts using Folin Ciocalteu's reagent as stated in the **Figure 4** and the result for the total phenolic content for the sample stated in **Table 2.**

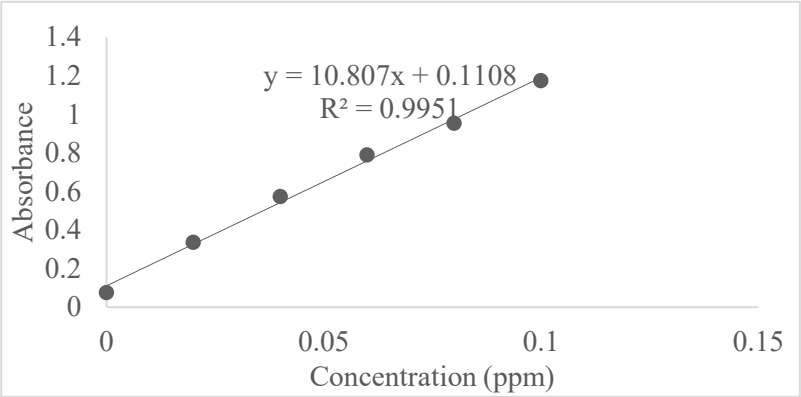

Figure 4: Standard calibration curve of gallic acid

Table 2: Total phenolic content of *P. volubilis* sample

Absorbance	GAE (ppm)
0.522	2.90

The results obtained from the **Table 2** for the total phenolic content of the *Malaysian P. volubilis* was 2.90 ppm and have been expressed as gallic acid equivalent (GAE). The result was lower than other reported value of *P. volubilis* oil from Peru (6.20 mg/100 g) [10] and from Xishuangbanna (6.50 ± 0.27 mg/100 g) [6]. This difference in values found maybe influenced by the difference between the geological factor such as surrounding temperature, moisture of the seed, and the nutritional content [10]. However the result can be improved by avoiding minor errors that can effects the accuracy of the results obtained.

3.3 Total Flavonoid Content (TFC)

Flavonoids are plant pigments that are responsible for plant color and have health-promoting properties due to their great pharmacological capabilities as reducing agents [11]. The total flavonoid contents was examined according to the development of a flavonoid-aluminium complex and expressed in terms of rutin equivalents (RE) (the standard cruve: y= 0.122x + 0.2574; R2=0.9366) as stated in **Figure 5** and the result was stated in **Table 3**.

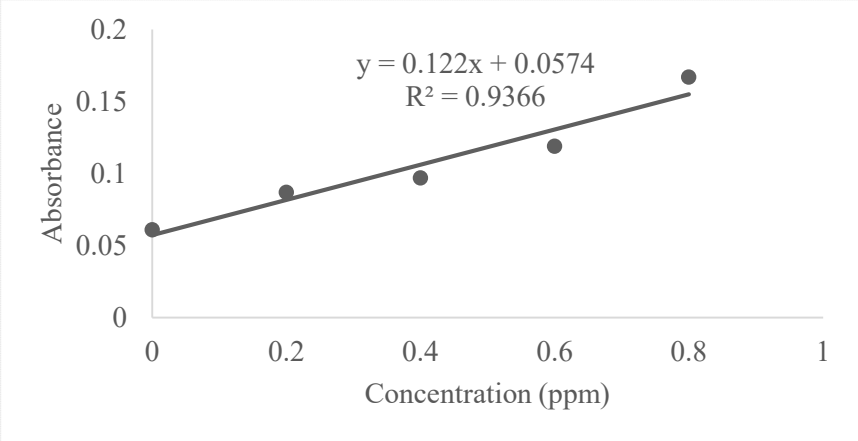

Figure 5: Standard calibration curve of rutin

Table 3: Total flavonoid content of the *P. volubilis*

Absorbance	RE (ppm)
0.094	2.50

Table 3 shows that the total flavonoid content of the *P. volubilis* sample was 2.50 RE ppm. The result obtained was lower than other reported value which is stated that the total flavonoid content value was 466.38±1.56 mg/g [11]. The variance in values discovered is due to the different extraction methods used.

4. Conclusion

As general conclusions, the antioxidant activity of *Malaysian P. volubilis* from its extract was investigated. These investigations revealed that this plant has the potential to be a source of safer natural antioxidants based on the higher result of total phenolic content (2.90 GAE ppm) and total flavonoid content (2.50 RE ppm). The IC_{50} showed the high value (4405.96 ppm), but because of the complexity of the composition of the extracts, more research is needed and conducted to get the optimum condition using other recorded data and to determine if the processing factors have an influence on the molecular nature and integrity of the antioxidant activity.

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